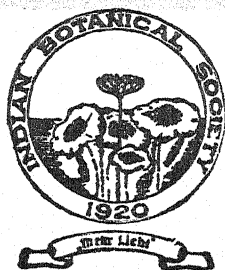


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of
The Indian Botanical Society
(Formerly "The Journal of Indian Botany")

EDITED BY
M. O. P. IYENGAR



VOL. XXV
1946

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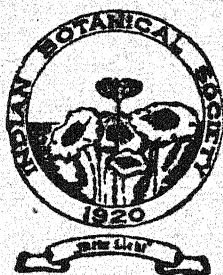
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VOL. XXV

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The Journal of the Indian Botanical Society

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VOL. XXV]

FEBRUARY, 1946

[No. 1

A NOTE ON THE ORIGIN AND NATURE OF THE STARCH SHEATH IN *HERACLEUM* STEM

BY GIRIJA P. MAJUMDAR

Department of Botany, Presidency College, Calcutta

Received for publication on August 15, 1945

INTRODUCTION

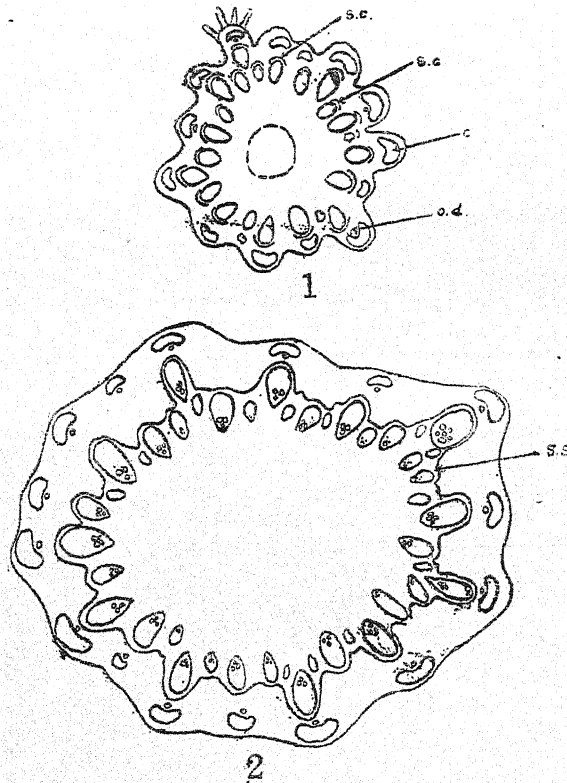
THE innermost layer of cortical cells, which is normally derived from the periblem and which encloses the pterome in the form of a sheath, has been described by Sachs¹⁸ as the *pterome sheath*. Strasburger (1930) has named this layer *phlæoterma*. The pterome sheath differentiates in two principal forms, viz., (1) as the *endodermis* with the characteristic structure of its walls, and (2) the *starch sheath*. Schoute (1902), however, uses all the three terms, viz., *endodermis*, *stärkescheide* (starch sheath) and *schuttscheide* (protective sheath) in the description of the stems of angiosperms in his treatise on the Stelar Theory.

The starch sheath, as defined by de Bary (1884), comprises a single layer of cells, which agrees with endodermis only in the close lateral connection of its elements, but differs from the latter in the absence of Casparian strips and other wall characteristics. The cells of the starch sheath, when the latter is well defined, differ from the neighbouring cells by permanently containing small but movable starch grains in abundance (p. 414). In many cases, however, de Bary points out, the two forms are mutually replaceable even in the same region of allied plants. Thus the pterome sheath is found as a starch sheath in the hypocotyledonary stem of *Helianthus annuus*, but as endodermis in the same region of *Tagetes patula* (p. 415).

Endodermis is a conspicuous and constant feature in the roots of all vascular plants with the exception of the Lycopodiaceæ¹²; it is present in the stems of pteridophytes, in the leaves of gymnosperms, in the rhizome of land plants and in the stems of water plants²⁴; but it is mostly absent in the stems of gymnosperms, in the aerial stems of angiospermous land plants and their leaves. Starch sheath, on the other hand, is found in the stems of most dicotyledons; but in stems

with abundant starch reserve in cortical cells the starch sheath as a layer is less conspicuous. Schoute (1902) observes that starch grains may be present in all the cells of the cortex, or in the innermost two or three layers, or only in the innermost layer of cells abutting on the stelar region. It is only in the last case that one should call the layer a starch sheath.

In most adult stems where starch sheath is developed, the layer appears in transverse section as a complete ring, e.g., *Ricinus*, *Helianthus*, *Tagetes*, etc., but in some cases, such as *Brassica oleracea*, it is incomplete and is found only opposite the leaf-trace bundles; and in *Aliangene alpina*, only opposite to the medullary rays of the central cylinder (de Bary, 1884, pp. 414-416).



Figs. 1-2. *Heracleum sphondylium*. Transverse sections of the stem, young and old. Fig. 1. Starch sheath as starch mantles (S.C.) surmounting each trace bundle. $\times 22.5$. Fig. 2. Starch sheath (S.S.) as a continuous but wavy ring in a slightly older axis than in Fig. 1. c., collenchyma strand; o.d., oil duct. $\times 30$.

In most of the text-books on plant anatomy 'starch sheath' and 'endodermis' have been used synonymously. Priestley and Scott (1939) state that the cells of the starch sheath by losing their starch grains undergo a different type of development to become an endodermis

(p. 389, cf. also Datta, 1945). Joshi (private communication) thinks that endodermis and the starch sheath belong to the same morphological category and the latter even without the Casparian strips can be called an endodermis. In my examination of a large variety of material, although such examination was often casual, I do not remember to have seen a typical starch sheath with Casparian strips, nor have I seen a typical endodermis by subsequently losing its Casparian strips and storing starch grains in its cells becoming transformed into a starch sheath, though there are reported cases where endodermis in stem has been found to have stored starch grains (de Bary, 1884, p. 125; Bower, 1935, pp. 42, 44, 56).

Both starch sheath and endodermis are usually regarded as the innermost layer of cortex [Sachs¹⁸, de Bary (1884), Strasburger (1930), Haberlandt (1914), Solereder (1908) and others], but Eames and MacDaniels (1925) describe it as the outermost layer of the stele (p. 105). Stellar endodermis has been noted in *Selaginella*,² *Pteris*⁶ and *Lycopodiaceae*.²² The starch sheath is described as one layer thick, but in jute, *Heracleum* and *Coccinea*, at some places, it is more than one layer in thickness, whereas the endodermis has, so far as I know, never been reported to be more than one layer in thickness, nor a starch sheath has ever been reported in roots proper. In *Equisetum*,²² however, the root lacks pericycle, and in its place an endodermis has been noticed which is two cell layers thick, the inner functioning as pericycle during the origin of secondary branches (p. 240).

Extensive works on the origin, development, structure and function of the endodermis have been done or discussed by Sachs (1875), Kroemer (1903), Mager (1907), Caspary, Schwendener (1882), Eisecke, Bower (1920, 1920), Priestley (1922, 1926), Priestley and North (1922), Isabel Soar (1922) and others; but not much developmental work, it appears, has been done on the origin and nature of the starch sheath in the stems of vascular plants. In this paper its origin and differentiation has been followed in *Heracleum sphondylium*. The observations recorded are based on serial hand sections of the growing points mounted in iodine solution.

OBSERVATIONS

The starch sheath in *Heracleum sphondylium* is first differentiated in connection with each leaf-trace bundle in the foliar primordium. It forms a crescent-shaped mantle (starke-sicheln⁹) surmounting the phloem portion of each adult bundle. In its origin and development each starch mantle is differentiated from the innermost layer of 4-6 layered parenchymatous tissue developed between each vascular bundle and its associated primary oil duct. Developmental studies show that this isolated starch mantle is procambial in origin. When the leaf bundles enter the axis, the leaf being sheathing at the insertion, the trace bundles, 15-16 in number, spread around the stem in the form of a ring, and the starch sheath in the form of isolated mantles is easily followed in the stem where it is seen most characteristically associated with each leaf-trace bundle of the primordium just inserted on the axis, whereas they cannot be traced opposite the trace bundles

of the primordia of the upper leaves, these trace bundles being mostly in the desmogen stage. At this early stage, therefore, the starch sheath is procambial in origin, differentiates in association with each leaf-trace bundle and stores starch grains in its cells perhaps for the supply of formative material to the dividing and differentiating procambial cells. If a transverse section of the axis at the level of insertion of the primordium is examined in iodine solution, the starch sheath instead of forming a continuous layer round the axis appears as so many crescent-shaped mantles of the different bundles (Fig. 1). It is only at a later period that the isolated starch mantles of all the leaf-trace bundles at different depths (from the surface) in the axis join together and form a continuous but wavy sheath of the adult axis which has been noted and described by previous workers (Fig. 2).

DISCUSSION AND CONCLUSION

In the vascular plants we come across at least three different kinds of sheaths in three well-defined regions, viz., *endodermis* in roots, *starch sheath* in stems, and *parenchymatous sheath* in leaves, besides the sclerenchymatous sheath of the monocotyledon bundles. They occupy almost identical position with regard to the vascular system; i.e., they limit the stele or bundles on the outside in the form of a cylindrical sheet, one layer thick and in close lateral connection of its elements. Each kind, it appears, has a definite function to discharge in its proper place, and structurally or by nature each is fitted for the purpose. Endodermis is sometimes seen to replace starch sheath in stem, or border parenchyma in the leaves. Miss Soar (1922) who made an extensive study on the structure and function of the endodermis in the leaves of Abietineæ concludes that the role of the endodermis in these leaves is contributory to the xeromorphy of that organ. The same might be said of the presence of the endodermis in the stem. Whenever a control is to be put on the lateral passage of water and solutes from the conducting region a primary endodermis is likely to develop in the region of the starch sheath in the stem. This has been well established by Datta (1945) in the transition from much branched vegetative region to the unbranched flowering axis of *Leonurus sibiricus* L.

The origin of endodermis and starch sheath also appears different. Endodermis always takes its origin in the layer of cells immediately outside the central cylinder, or the vascular bundles as the case may be. The starch sheath on the other hand, in the cases studied, invariably originates in close association with each trace bundle in the form of *stärke-sicheln* which finally unite to form the continuous layer found in the adult axis. The starch mantles, however, may not unite at all, so they remain free opposite each leaf-trace bundle, as in *Brassica oleracea*, or they store starch grains only in the cells opposite medullary rays, where two contiguous mantles meet, as seen in *Aliangene alpina*. Interrupted starch sheath in various forms has been reported by Haberlandt (1914). The endodermis is always circular in outline, but the starch sheath is wavy. This wavy nature of the starch sheath layer points to its procambial origin, as has been demonstrated in *Heracleum*.

Hence it is concluded that in the case studied no morphological value of a permanent nature can be attached to the starch sheath as a layer in the same sense as in the case of endodermis, and as such the two terms should not be used synonymously.

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SOME ABNORMAL FLOWERS OF *ANGELONIA GRANDIFLORA*

BY C. VENKATA RAO

Hindu College, Guntur

Received for publication on February 21, 1945

Angelonia grandiflora C. Morr. is a member of the family Scrophulariaceae and a native of S. America. It is a common border plant in the gardens of South India and other parts of this country. The plants are generally propagated by cuttings, as the flowers ordinarily fail to produce viable seeds. The flowers are of a purplish colour. Last year, however, while visiting a local garden, the author came across some flowers of this species which, instead of being coloured, were green like the foliage leaves. On closer observation, these flowers were seen to show several abnormalities. These are briefly reported here.

The normal flowers of *Angelonia grandiflora* show the usual Scrophulariaceous structure. Fig. 1 illustrates one of them. They are borne in a solitary and axillary fashion, and possess a long slender pedicel. The calyx consists of five, free, very small sepals. Each sepal measures only about or less than two millimetres. The corolla has a short narrow tube, which expands above into a cup-shaped structure with five lobes spreading out in a bilabiate manner. The four epipetalous stamens stand towards the posterior part of the corolla. The globose ovary is topped by a short style, that does not project beyond the corolla tube.

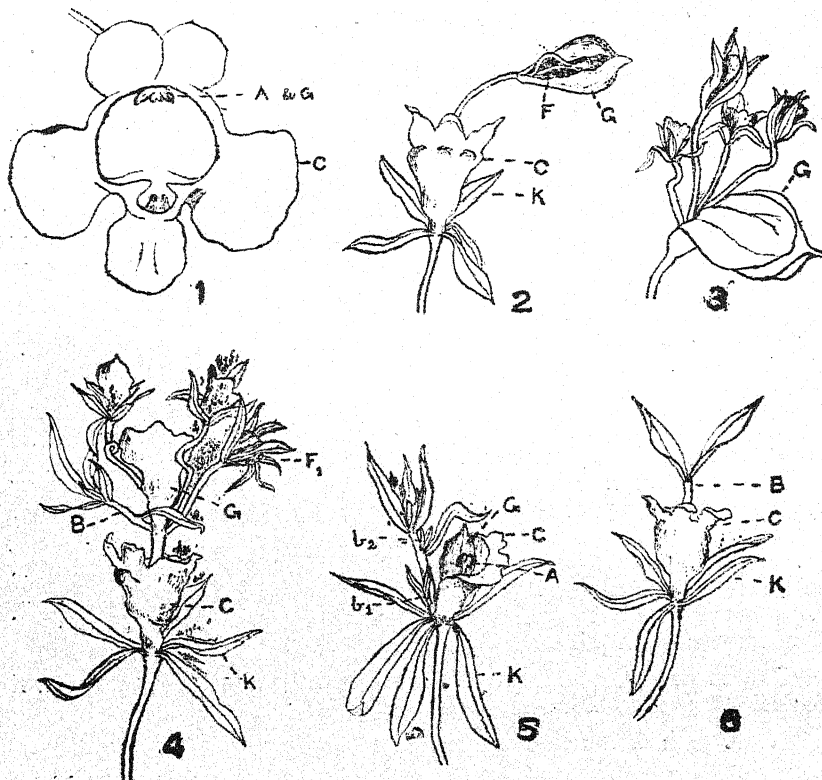
The author collected two branches bearing about forty abnormal flowers. The usual abnormalities in these flowers are :

1. A general increase in size.
2. Enlargement of the sepals into large foliaceous structures sometimes measuring up to 2 cm. in length.
3. Enlargement of the corolla tube into a longer and wider structure. Green colour of the petals.
4. Development of a long gynophore below the ovary, as recorded by Joshi (1933 and 1939) in *Argemone mexicana*.
5. Considerable increase in the size of the ovary.
6. Much proliferation.

The stamens do not appear externally to have undergone much change.

Fig. 2 illustrates a typical abnormal flower and clearly shows the abnormalities Nos. 1-5 listed above. Further, the ovary shows a small

slit on one side near the base, revealing the presence of some stalk-like structures within. On opening the ovary (Fig. 3), these are seen to be the pedicels of four flowers arising from its base. These flowers are still in the bud stage and are enclosed by the ovary wall, but as they develop, the ovary wall bursts open and they come out. This is seen in Fig. 4. Such proliferation of flowers from the ovary of a parent flower is very common and was observed in most of the flowers. Generally four



Figs. 1-6. *Angelonia grandiflora*.—Fig. 1. A normal flower. Figs. 2 and 4-6. Flowers with various kinds of abnormalities. Fig. 3. The flower shown in Fig. 2 with the ovary split open. K, calyx; C, corolla; A, androeium; G, gynoeium; F in Fig. 2, stalk of a flower arising inside the ovary; F1 in Fig. 4, a flower with seven sepals; b₁ and b₂ in Fig. 5, axillary buds on the vegetative shoot arising from the axil of the corolla of a flower; B in Fig. 6, the vegetative shoot arising from the apex of the thalamus in place of the gynoeium. For further explanation see text.

flowers come out of one ovary, but in some cases only two and in one case five have been noticed. In all such ovaries the septum is wanting so that the ovary becomes unilocular. In a few others which do not develop the flowers or branches inside, the ovary expands merely into a hollow structure with a thin septum but in no case were any ovules found.

Fig. 4 shows one further peculiarity. Of the four shoots coming out of the ovary, one (marked B in the figure) has developed into a vegetative branch with about 8 leaves. The other three have formed flowers. One of these flowers (F_1) shows seven sepals instead of the customary five.

Fig. 5 represents a flower showing the development of a vegetative shoot from the axil of the corolla. Such variation was observed in six flowers. The accessory shoot in Fig. 5 has eight well-developed leaves, the basal two of which already show distinct axillary buds (b_1 and b_2). No branches arise in such cases from the ovary.

Fig. 6 shows the only specimen observed in which a vegetative shoot instead of the gynœcium arises from the apex of the thalamus.

The author takes this opportunity to express his gratitude to Dr. A. C. Joshi for his help in the preparation of this note.

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GERMINATION OF THE HETEROCYST IN
TWO MEMBERS OF THE RIVULARIACEÆ,
GLOEOTRICHIA RACIBORSKII WOLOSZ.
AND *RIVULARIA MANGINI* FREMY*

BY T. V. DESIKACHARY, M.Sc.

University Botany Laboratory, Madras

Received for publication on September 15, 1945

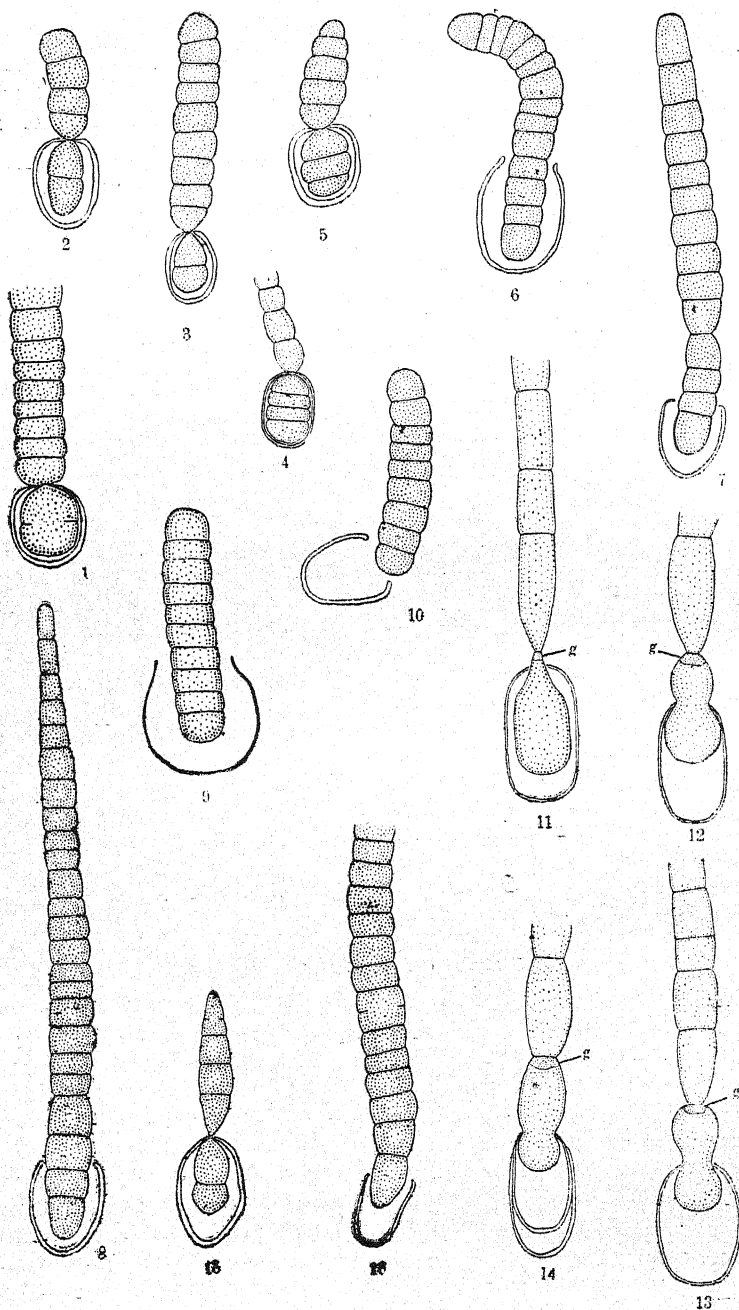
USUALLY heterocysts do not germinate. But in a few genera they have been observed to germinate. Even here it is not a very common or regular feature. Germination of the heterocyst has been recorded so far in four species of *Nostoc*, viz., *N. commune* Vaucher (Geitler, 1921), *N. ellipsoforum* (Desm.) Rabenhorst (Geitler, 1921), *N. Linckia* (Roth) Bornet (Geitler, 1921) and *N. microscopium* Carm. (Geitler, 1921), and two species of *Anabæna*, viz., *A. variabilis* Kützinger (Geitler, 1921) and *A. steloides* Canabæus (Canabæus, 1929), two species of *Tolypothrix*, viz., *T. lanata* Wartmann (Geitler, 1921) and *T. Elenkinii* Hollerbach (Hollerbach, 1928), and one species of *Calothrix*, viz., *C. Weberi* Schmidle (Steinecke, 1931). Iyengar and Desikachary (1944) have recorded a case of germination of the heterocyst in *Brachytrichia Balani* (Lloyd) Born. and Flah. Formation of gonidia by the heterocyst has been recorded by Brand (1901) in *Nostoc commune* Vaucher and *N. microscopium* Carm. and by Spratt (1911) in *Anabæna cycadeæ* Reinke. The gonidia formed by the heterocyst later on develop into new filaments. Thus the germination of the heterocyst is known in only five genera, viz., *Nostoc*, *Anabæna*, *Tolypothrix*, *Calothrix* and *Brachytrichia*. The writer came across the germination of the heterocyst in two genera, viz., *Gloeotrichia* and *Rivularia*. A detailed account of the germination is given below :—

Gloeotrichia Raciborskii Wolosz.

Gloeotrichia Raciborskii was collected from a lake in Chingleput, in the month of January 1942. A portion of the material was preserved in 4% Formalin on the spot. The remaining portion of the alga was brought to the laboratory in the living condition and kept growing in the laboratory in the lake water to which some sterilized Schreiber's nutrient solution was added.

The material was vernalized several times at different temperatures ranging from 32° F. to 50° F. and for varying periods ranging from a few hours to three days, and then left at room temperature for a number of days. No heterocysts were observed to germinate for quite a long time. Finally, in one of the attempts, a number of heterocysts were

* This paper formed part of a thesis approved for the Master of Science Degree of the Madras University.



Text-figs. 1-16.—Figs. 1-14. Stages in the germination of the heterocyst in *Glæotrichia Raciborskii* Wolosz. Fig. 1. Beginning of the first division of the

contents of the heterocyst. Figs. 2 and 3. Two-celled germling stages. Fig. 4. Four-celled stage. Fig. 5. Three-celled stage. Fig. 6. A long germling that has grown out of the heterocyst. Figs. 7 and 8. Long germlings that have grown out of the heterocyst connected with the old trichome. Fig. 9. A ten-celled germling with traces of the old heterocyst wall still persisting at the base. Fig. 10. Germling fully come out of the old heterocyst wall. Figs. 11-13. Various stages of the coming out of the contents of the heterocyst without undergoing any division (g = granule). Fig. 14. Heterocyst rejuvenating for a second time. Note the two heterocyst walls. Figs. 15 and 16. Stages in the germination of the heterocyst in *Rivularia Manginii* Frey. Figs. 1, 2, 3, 10, 15, $\times 1150$; Figs. 5-9, 16, $\times 1100$; Figs. 11-14, $\times 1450$; Fig. 4, $\times 800$.

found to have germinated. In this case the material had been exposed to a temperature of 45° F. for a period of 24 hours and then left at room temperature. This material was kept under observation and all the details of germination up to the fully developed trichome were carefully followed.

The heterocyst in the alga is situated at the base of the filament, and has a single pore on the trichome side. A large granule is present very near the pore.

Germination of the heterocyst.—The contents divide into two cells (Text-figs. 2 and 3; Pl. I, Fig. 2). The beginning of the cross-wall formation can be seen in Text-fig. 1 and Pl. I, Fig. 1; and in Text-figs. 2 and 3 and in Pl. I, Fig. 2 the division into two cells is complete. The granule present near the pore of the heterocyst evidently disappears at the commencement of the division (Text-fig. 1; Pl. I, Fig. 1). By further division of both these cells a four-celled germling is formed (Text-fig. 4; Pl. I, Fig. 3). Sometimes only one of the two cells divides and then only three cells are seen in the germling (Text-fig. 5; Pl. I, Fig. 5). As the germling grows longer and emerges out through the pore, it is seen still connected with the rest of the trichome (Text-figs. 7 and 8; Pl. I, Fig. 4). Whether, after growing further, this germling will break away from the old trichome and become an independent trichome or will continue to grow attached to the old trichome could not be definitely stated from the stages of germination available in the material. But a few cases of well grown germlings with the old heterocyst wall still persistent at the base were found in the material (Text-figs. 6, 9 and 10; Pl. I, Fig. 6). These well grown germlings were quite independent and were not connected with the old trichome portions. These germlings might have broken away from the old trichome or might merely be the product of the germination of isolated heterocysts.

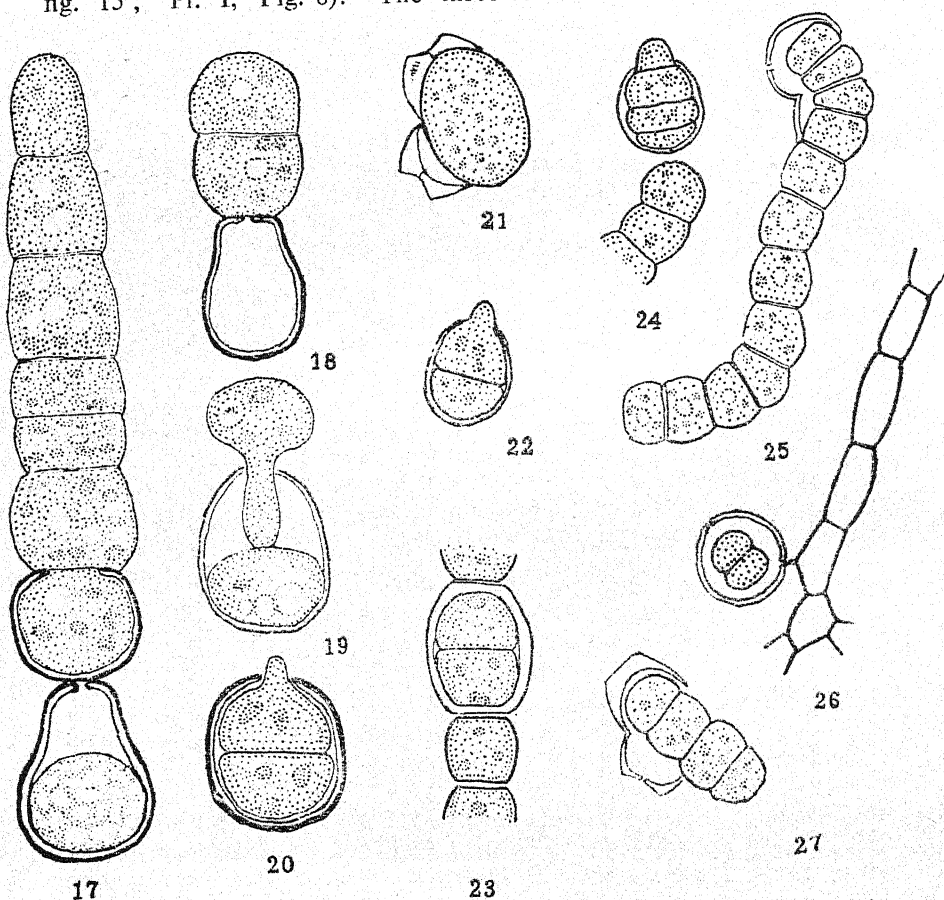
During the germination of the heterocyst the inner cellulose layer of the heterocyst wall is the first to be dissolved and the outer layer gets dissolved away later on. This outer layer is generally seen persisting for quite a long time at the base of the germling trichome (Text-figs. 7-10).

In the living material which was brought to the laboratory the entire contents of the heterocysts were sometimes observed to come out of the heterocyst wall gradually without undergoing any division (Text-figs. 11, 12 and 13) and were still connected with the rest of the trichome. The contents which have come out of the old wall secrete

a new wall and become a heterocyst again. This evidently represents only a case of rejuvenation of the contents of the heterocyst and not of germination. In one case the contents of the heterocyst have rejuvenated twice. After coming out of the wall a short distance the contents evidently surrounded itself with a new wall and was coming out of the new wall again (Text-fig. 14; Pl. I, Fig. 7).

Rivularia Mangini Frey

This alga was collected on moist rocks near a waterfall at Tirupati in South India. This collection which was preserved in 4% formalin on the spot proved interesting since a few germinating heterocysts were found in it. As in the case of *Glæotrichia* described above, a two-celled germling was found enclosed inside the heterocyst wall (Text-fig. 15; Pl. I, Fig. 8). The three-celled or four-celled germling



Text-figs. 17-27.—Figs. 17-20. Stages in the germination of the heterocyst in *Calothrix Weberi* Schmidle (after Steinecke). $\times 1900$. Figs. 21-25 and 27. Stages in the germination of the heterocyst in *Nostoc commune* Vaucher (after Geitler). Fig. 26. A two-celled germling in a heterocyst in *Brachytrichia Balani* (Lloyd) Born, and Flah. (after Iyengar and Desikachary).

stages were not found in the material. But a few long germlings were found with traces of the old heterocyst wall still persisting as a cap at the base. These are cases of germination of the heterocyst under natural conditions, since the material was not treated in any way in the laboratory but was preserved as soon as collected. The germination of the heterocyst in *Brachytrichia Balani* recorded by Iyengar and Desikachary (1944, p. 46, Fig. 7 i) was also a case of germination under natural conditions (Text-fig. 26).

DISCUSSION

Geitler (1921), Hollerbach (1928), Canabæus (1929) and Steinecke (1931) have recorded the germination of the heterocysts in several blue-green algæ. In all these cases they found that the contents by a series of transverse divisions became a germling. In most of these cases only a few divisions of the contents were followed. But all the various stages of germination of the contents and the development of the germling into long filaments appears to have been followed so far only in three members of the Cyanophyceæ, viz., *Nostoc commune* (Geitler, 1921), *Tolypothrix Elenkinii* (Hollerbach, 1928) and *Calothrix Weberi* (Steinecke, 1931).

According to Geitler in *Nostoc commune* the contents of the heterocyst first divide to form a two-celled germling (Text-figs. 22 and 23) which by further division becomes a four-celled germling (Text-figs. 24 and 27). This four-celled germling becomes free either through a circumcissal break of the heterocyst wall at the middle into two pieces (Text-figs. 25 and 27) or comes out through one of the pores (Text-figs. 22 and 24). The germling after coming out, by further division, grows into a filament.

The stages of germination of the heterocyst in *Calothrix Weberi* (Text-figs. 17-20) as recorded by Steinecke (1931) are somewhat different from those recorded by Geitler in *Nostoc commune*. The contents of the heterocyst in *Calothrix Weberi* by a transverse division form a two-celled germling (Text-fig. 20). The cell close to the pore of the heterocyst squeezes itself out of the heterocyst wall through the pore (Text-figs. 19 and 20) and then by further divisions gradually grows into a mature filament (Text-fig. 17). The second cell which remains inside degenerates (Text-figs. 17 and 19).

In the two algæ dealt with in this paper not even a single case was observed of the emerging out of the upper cell to form a new filament and the degeneration of the lower cell as recorded by Steinecke (1931) in *Calothrix Weberi*. The writer's observations on the germination of the heterocyst in *Glæotrichia Raciborskii* agree closely with those of Geitler on *Nostoc commune*. But the germling in *Gl. Raciborskii* does not emerge from the heterocyst wall through a break in the middle, but emerges out of the heterocyst wall always through the widening of the pore.

Finally no case of godidia formation by the heterocyst as recorded by Brand and Spratt was observed in the two present algæ.

SUMMARY

Germination of the heterocysts has been known only in five genera, viz., *Nostoc*, *Anabæna*, *Tolypothrix*, *Calothrix* and *Brachytrichia*. The germination of the heterocyst in two more genera, viz., *Glæotrichia* (*Gl. Raciborskii*) and *Rivularia* (*R. Mangini*) is described in the paper.

The details of germination agree closely with those recorded by Geitler in *Nostoc commune*. The stages of germination of the heterocyst described by Steinecke in *Calothrix Weberi*, viz., the emerging out of the upper cell of the two-celled germling and the degeneration of the lower cell, were not observed in the forms investigated here.

The author wishes to express his great indebtedness to Prof. M. O. P. Iyengar, M.A., Ph.D. (Lond.), F.L.S., for his constant guidance and help during the course of this investigation and in the preparation of this paper. His sincere thanks are also due to the authorities of the University of Madras for the award of a research scholarship during the tenure of which the present investigation was carried out.

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EXPLANATION OF THE PLATE

Figs. 1-7. Stages in the germination of the heterocyst in *Gleotrichia Raciborskii* Wolosz.

Fig. 1. The beginning of the division of the contents of the heterocyst.

Fig. 2. Two-celled germling.

Fig. 3. Four-celled germling.

Fig. 4. A germling with traces of the heterocyst wall still present at the base growing still connected with the old trichome.

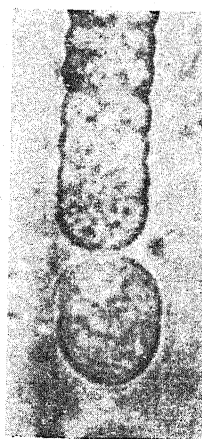
Fig. 5. Three-celled germling.

Fig. 6. A well-grown germling with the heterocyst wall covering the base.

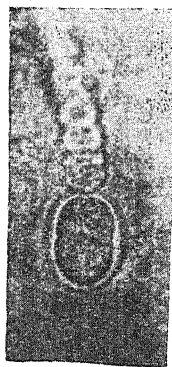
Fig. 7. Heterocyst rejuvenating for a second time. Note the two heterocyst walls.

Fig. 8. A two-celled germling surrounded by a brownish mucilage in *Rivularia Mangini* Frey.

(Figs. 1, 5, 7, $\times 1900$; Figs. 2, 3, $\times 1200$; Fig. 4, $\times 1100$; Figs. 6, 8, $\times 1650$.)



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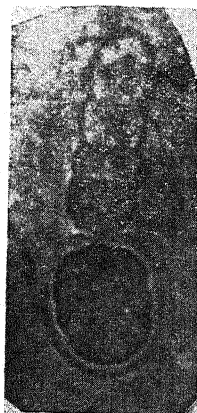
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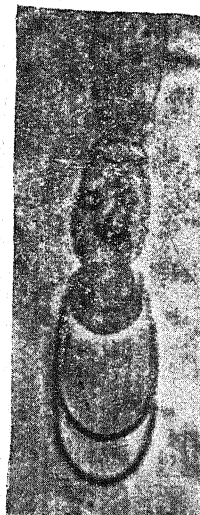
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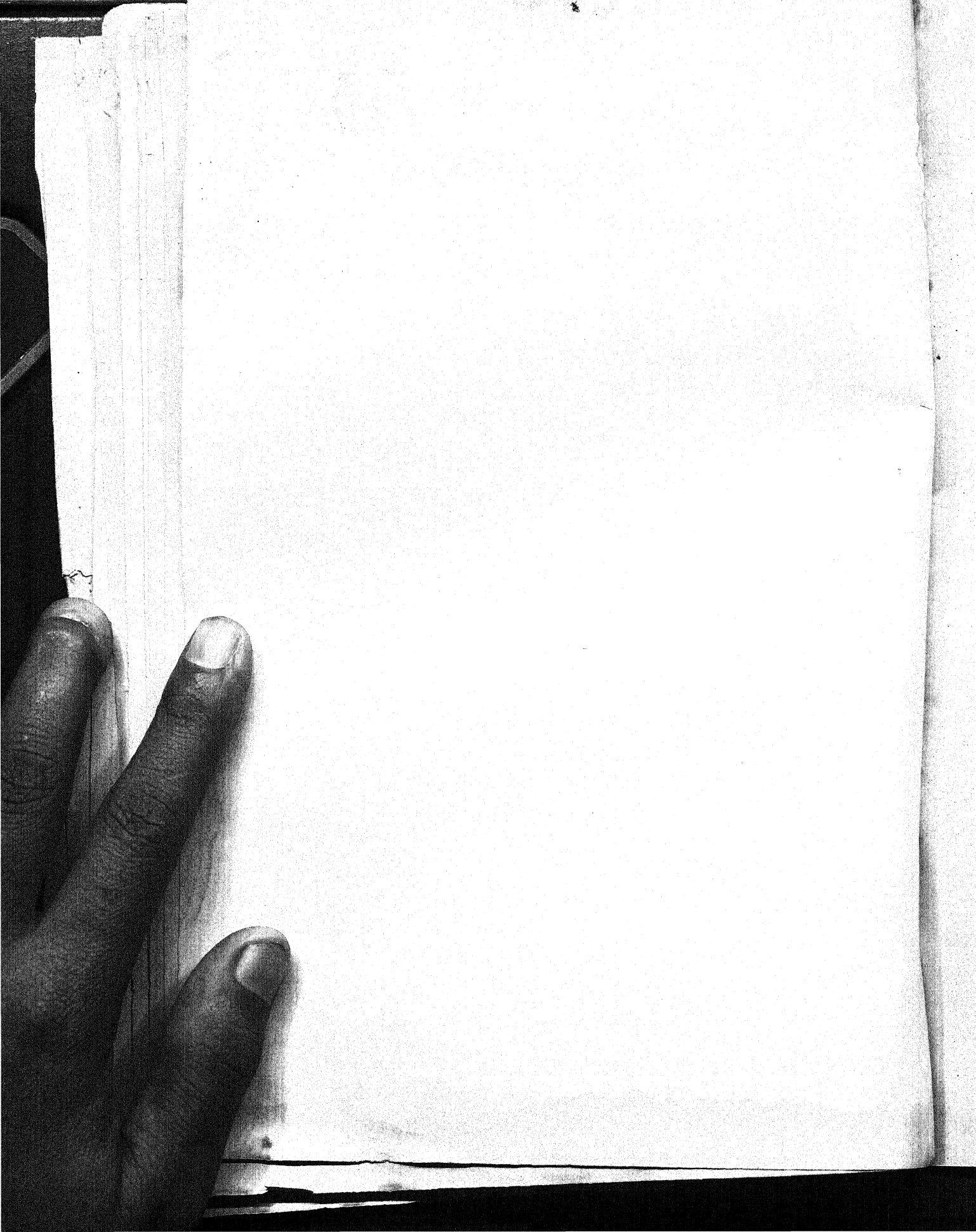
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T. V. DESIKACHARY—

*GERMINATION OF THE HETEROCYST IN GLÆOTRICHIA
RACIBORSKII WOŁOZ, AND RIVULARIA MANGINI FREMY*



NUCLEAR DIVISION IN *SPIROGYRA**

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INTRODUCTION

THE details of nuclear division in such a common alga as *Spirogyra* have formed the subject of repeated study by various workers. Strasburger (1875) was the first to investigate the process in the alga. Since then there has been quite a lot of discussion on the matter, the main points of discussion being the nature of the nucleolus and the origin of the chromosomes. A summary of the work done by the earlier workers on mitosis in *Spirogyra* is given by Mitzkewitsch (1898), Lutman (1911) and Wisselingh (1921).

Three views have been put forward so far, viz., (1) that all the chromatin material is lodged in the nucleolus and that the chromosomes therefore take their origin from the nucleolus (Strasburger, 1875; Tangl, 1882; Moll, 1893; Mitzkewitsch, 1898; and Berghs, 1906), (2) that the chromosomes are derived partly from the nucleolus and partly from the reticulum (Strasburger, 1882; Flemming, 1882; and Wisselingh, 1900, 1902, 1921) and (3) that the chromosomes do not originate from the nucleolus at all, but are derived solely from the reticulum (outer nucleus) (Strasburger, 1888; Degagny, 1894; Geitler, 1930, 1935 a, b; and Suematsu, 1936).

Strasburger (1875), from his investigation of the nuclear division in *Spirogyra orthospira*, stated that the nucleolus divides into a number of bodies which arrange themselves into an equatorial plate. But, later on in 1882 (pp. 162-75), after examining the nuclear division in *Spirogyra majuscula*, he modified his earlier view and stated that the nucleus has got a reticulum which forms the equatorial plate with the help of the substance of the nucleolus. Later on in 1888, after working on *S. polyteniata*, he changed his view still further and stated that the reticulum alone takes part in the formation of the chromosomes as in the higher plants.

Flemming (1882) considered that the nucleolus together with the reticulum contributes the material for the construction of the equatorial plate. The reticulum according to him contains an extremely meagre quantity of chromatin as compared with the large amount in the nucleolus.

Tangl (1882), Moll (1893) and Mitzkewitsch (1898) expressed the opinion that the equatorial plate is formed at the expense of the substance of the nucleolus which consists of both linin and chromatin.

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Meunier (1887) thought that the nucleolus contained all the chromatin and furnished the necessary material for the formation of the equatorial plate. He described the nucleolus as having an unbroken thread-like structure inside and stated that it possessed all the properties of the nucleus of other plants. He called the nucleolus a "*noyau en miniature*".

Degagny (1894-96) states that the equatorial plate is formed from the reticulum which at the beginning of mitosis closely envelops the nucleolus and the substance of the nucleolus passes into it.

Wisselingh (1900, 1902, 1921) states that the chromatin material is found both in the nucleolus and in the reticulum. By a progressive digestion of the nucleus in 40% chromic acid, he comes to the conclusion that two chromosomes arise from the nucleolus and the remaining ones from the reticulum.

Berghs (1906) states that twelve chromosomes arise from the nucleolus, the reticulum being quite free from it. In the nucleolus a second substance still remains which stains feebly and divides into two parts which move to the poles along with the chromosomes.

Merriman (1913) describes a spireme originating from the material derived from both the nucleolus and the reticulum. This spireme, she states, consists of a granular substance derived from the nucleolus and a filamentous material from the nuclear network. She later on, in 1916, however, states that the nucleolus does not fragment directly into chromosomes, but only contributes to the less dense substance seen at metaphase. She expresses the view that *Spirogyra* as regards the constitution and behaviour of its nucleolus need not be placed in a different category from the other green algæ or from the higher plants.

Stolley (1930) states that dark granular or thread-like structures appear in the outer nucleus, while the nucleolus is still intact, and that these structures are seen at the periphery of the nucleolus as it begins to break up. She thinks that it is probable that they are derived from the outer nucleus (reticulum) and get imbedded later on in the nucleolar substance. She considers these bodies as chromosomes and doubts whether the substance in which they are found could still be called the nucleolus. She states that a study of their chemical nature is necessary to decide their origin.

Geitler (1930) investigated three species of *Spirogyra* and found that the chromosomes in each of them are fully formed in the outer nucleus (the reticulum), while the nucleolus is still intact. He thinks that the chromosomes in the outer nucleus are masked by some substance for some time and become visible only later on. The duration of masking, however, is not the same in all species. He states that the mistaken view of the earlier authors that the chromosomes originate from the nucleolus is evidently due to this. Later on he (Geitler, 1935 *a*) investigated one more species (*Spirogyra X*) and confirmed his earlier view that the chromosomes arise in the 'outer nucleus' quite independently of the nucleolus. In the same year he (1935 *b*) investigated a number of other species by using Feulgen stain

and found that in every case the nucleolus in the resting nucleus remained unstained, while in the outer nucleus were found several deeply stained granules which he considered were probably chromocentres. The chromosomes, which are organised a little later in the outer nucleus, also show a positive reaction to the stain, while the nucleolus, which is still intact, shows a negative reaction. He comes to the conclusion that the chromosomes arise only from the reticulum and that the nucleolus does not contribute anything to their formation and finally expresses the view that the nucleus in *Spirogyra* is quite similar in structure and substance to that of the higher plants.

Conard (1933, 1939) states that the chromosomes are found in the outer nucleus and that the nucleolus breaks up into a granular mass which becomes a thick disc-like mass in which the chromosomes are found imbedded during metaphase.

Suematsu (1936) states that the nucleus of *Spirogyra* resembles that of the higher plants, that the chromatic granules appearing in the outer nucleus give rise to the chromosomes and that the chromosomes throughout the process of mitosis show a positive reaction to Feulgen stain.

In view of the different views expressed regarding the origin of the chromosomes, a detailed investigation of the nuclear division was taken up in a few species of *Spirogyra* occurring in Madras in order to find out how far the results obtained by the writer agreed with, or differed from, those obtained by the previous workers.

MATERIAL AND METHODS

Four species of *Spirogyra* were taken up for investigation, viz., *S. columbiana* Czurda, *S. Fuellibornei* Schmidle, *S. paraguayensis* Borge and *Spirogyra* sp. Of these, *Spirogyra columbiana* and *S. sp.* were growing in a water drain near the laboratory, *S. Fuellibornei* in a temporary pool in the Madras beach and *S. paraguayensis* in a pond inside the Madras Museum compound. The material was fixed at intervals of half an hour during the course of twenty-four hours. The most abundant division figures were obtained in material fixed between 11 p.m. and 1 a.m.

The following fixing fluids were tried:—Flemming's weak, Flemming's strong, Flemming's strong diluted with an equal amount of water, Nawaschin's fluid, Chamberlain's chromo-osmo-acetic mixtures, Schaudinn's sublimate-acetic alcohol and Bouin's fluid as modified by Allen (P.F.A.). Of these, Bouin's fluid and Nawaschin's fluid gave the best results.

The material fixed in Nawaschin's fluid was washed in running water for six to eight hours and then taken up through the alcohol grades to 70% alcohol. And the material fixed in Schaudinn's solution was washed in 50% alcohol until it was quite free from mercuric chloride, and then taken up to 70% alcohol.

For imbedding in paraffin the following procedure was adopted. A small bunch of the filaments in 70% alcohol was taken up by one

end with a pair of forceps, when the filaments hang down more or less parallel to one another. Small lengths of this bunch of filaments were cut with a pair of scissors and rolled up in a small piece of lens-paper. These tiny lens-paper rolls with the material inside were treated as whole materials and passed through the alcohol and the xylol grades and finally infiltrated in paraffin. After infiltration, the lens-paper was carefully removed from the bundle of filaments inside with the aid of hot needles and the material was finally imbedded. Sections 5–10 μ thick were cut with the aid of a Spencer rotary microtome.

Whole-mount-preparations in Venetian turpentine (Chamberlain, 1933, p. 106) and in Canada balsam (McClung, 1937, pp. 202–03) were also made from material in 70% alcohol. The sections, as well as the whole-mount-material, were stained in Heidenhain's iron-alum hæmatoxylin. One species, *S. Fuellibornei*, was stained in Feulgen stain also. For this purpose, the material in 70% alcohol was taken down to water and washed thoroughly, rinsed in distilled water and hydrolysed in N.HCl at 60° C. for about 6–10 minutes, then rinsed two or three times in cold N.HCl and finally in distilled water before transferring to the stain, where it was kept for 6 hours to overnight. The stained material was not washed in sulphurous acid, as the latter destained it very rapidly.

Spirogyra columbiana CZURDA

The resting nucleus.—The resting nucleus in this species is elliptic to spherical in shape (Text-fig. 1). It has a large darkly staining nucleolus with a crisp outline. The outer nucleus is occupied by a faintly stained delicate reticulum. The nucleolus shows one or more vacuoles inside it (Text-fig. 1; Pl. II, Fig. 3).

Prophase.—At the beginning of the prophase, the nucleus enlarges slightly and becomes elongated along the length of the cell. The nucleolus is still quite intact and shows a sharp outline. Darkly staining granules are seen in the outer nucleus in the meshes of the reticulum (Text-fig. 3). At this stage the nucleolus becomes somewhat irregular in shape and loses its outline. At a slightly later stage, short rod-like chromosomes are seen scattered in the outer nucleus (Text-fig. 4). The surface of the nucleolus appears somewhat broken up. At a still later stage the nucleolus completely loses its sharp outline and becomes a granular mass, and some of the rod-like chromosomes appear with their ends half immersed inside the nucleolar substance (Text-fig. 5). The chromosomes are found later on completely imbedded inside the greyish granular nucleolar substance. Each of these chromosomes is surrounded by a thin hyaline outer portion. This hyaline area is seen very clearly round the chromosomes which are imbedded in the greyish nucleolar substance (Text-fig. 6).

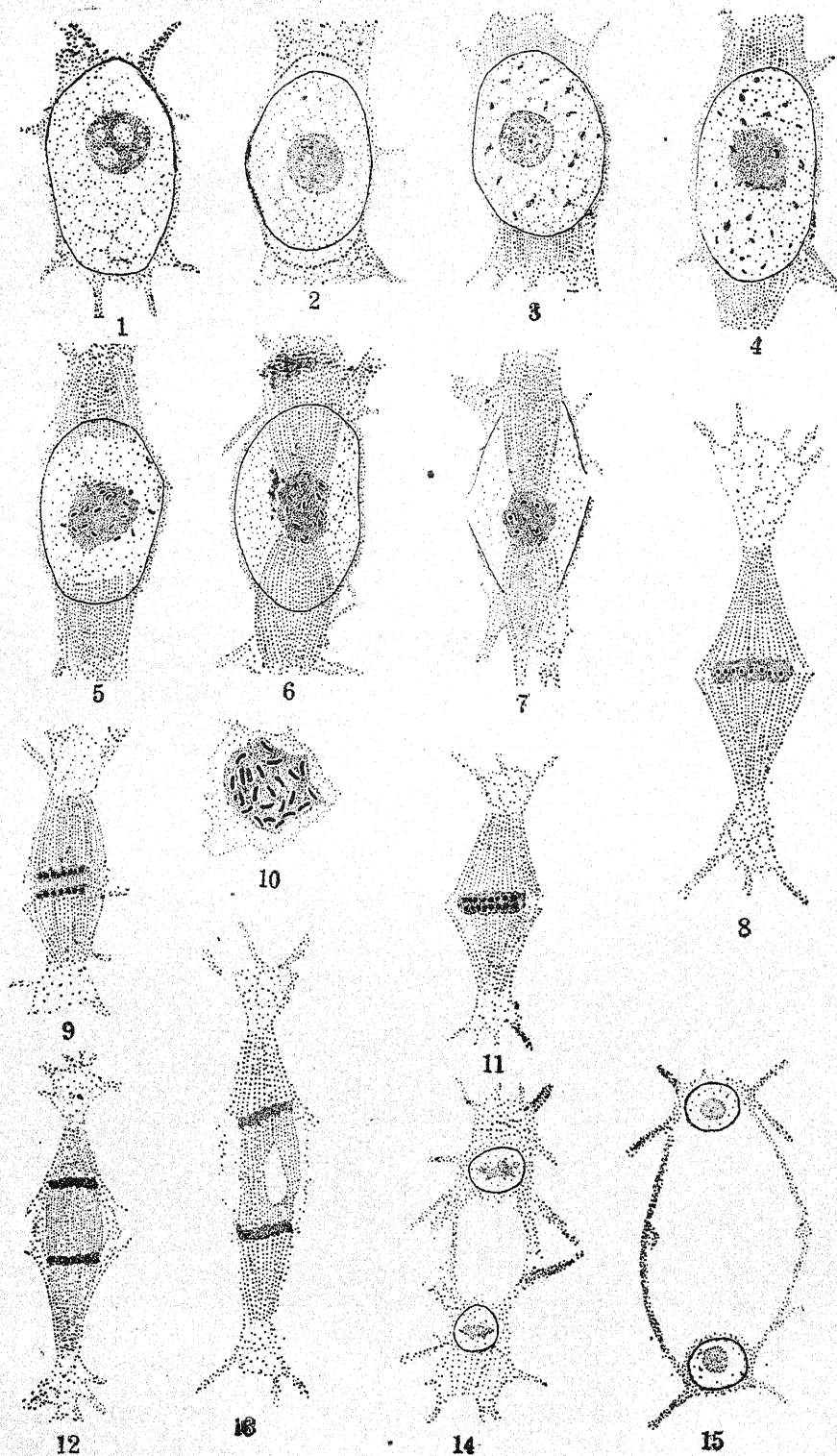
At the beginning of the prophase, a dense cytoplasmic accumulation is seen at each polar region of the nucleus (Text-fig. 2). This accumulation is at first spongy, but later on a definite transparent region could be seen between the nuclear membrane and the rest of the cytoplasm at each end of the nucleus. These two transparent

regions are crescent-shaped and appear to be the polar caps (Text fig. 2). A little later, striations which are very delicate and parallel to one another, appear in these polar caps and extend outwards and away from the nuclear membrane (Text-fig. 3). McAllister (1931, p. 840) found that the nuclear membrane in *S. setiformis* at this stage is depressed at the polar regions. He states that Berghs (1906) interprets it as due to the pushing action of the fibres of the caps. No such depression was seen at the polar regions in the present alga. After this stage the striations gradually extend inside the nucleus (Text-fig. 5), and as they approach the nucleolar mass, converge a little and become finally closely attached to the nucleolar mass (Text-fig. 6). The fibres outside the poles of the nucleus are now longer than before showing that they have extended still further into the cytoplasm. The nuclear membrane then appears to 'break down' at the two poles at the region of the exit of the striations. At this stage, in median optical section, the nuclear membrane is completely absent at the poles, though it is still quite distinctly visible in the remaining portions. After sometime the nuclear membrane becomes broken up at the sides also, the break starting first at the equatorial region (Text-fig. 7). At this stage the nuclear figure is quite long with the spindle fibres well developed. The facts detailed above would appear to suggest that the spindle fibres originate in the cytoplasm of the polar cap portion and then extend into the nuclear cavity.

Metaphase.—The nucleolar substance with the chromosomes inside it becomes flattened and assumes the form of an equatorial plate in which the chromosomes are arranged very regularly (Text-fig. 8). The nucleolar substance stains grey with iron-alum hæmatoxylin, while the chromosomes take up a dark stain. In polar view of the plate, the chromosomes appear rod-shaped and are closely arranged. The thin hyaline area mentioned already is seen round each chromosome (Text-figs. 8, 9). The number of chromosomes seen in polar views of the metaphase plate obtained in transverse sections of the filaments is 24 (Text-fig. 9). By this time the nuclear membrane completely disappears and no trace of it is seen either in the polar or side view of the spindle.

Some of the outer fibres of the spindle at this stage, are seen broken at the equatorial region (Text-fig. 8), while the remaining ones are continuous. The spindle fibres converge towards the pole. They do not, however, meet at a single point, but end in a more or less round cytoplasmic aggregation from which strands of cytoplasm radiate outwards (Text-figs. 8, 9, 11, 12). The main central portion of the aggregation is somewhat diffuse and does not take up much stain. A few darkly stained granules are seen inside it. These polar cytoplasmic aggregations show a distant resemblance to asters of a nuclear spindle, but no centrosomes are seen in them.

Anaphase.—At the beginning of anaphase the chromosomes divide and are arranged in two parallel rows within the nucleolar matrix (Text-fig. 11). The nucleolar plate then splits transversely into two, and the two plates, each with its own set of daughter-chromosomes imbedded in it, soon separate and begin to move towards the poles of



Text-figs. 1-15. *Spirogyra columbiana* Czurda.—Fig. 1. Resting nucleus. Note vacuoles inside the nucleolus. Fig. 2. Polar caps formed at the two poles

of the nucleus. Fig. 3. Chromosomes originating in the outer nucleus. Note the nucleolus still intact with a regular outline; striations developed in the polar cap region. Fig. 4. Chromosomes distributed in outer nucleus and the outline of the nucleolus already broken. Fig. 5. Most of the chromosomes imbedded in the nucleolar mass with a few still outside. Note the spindle fibres extending into the nuclear cavity from the polar regions. Fig. 6. Late prophase. All the chromosomes imbedded in the nucleolar substance and the spindle fibres extended up to the nucleolar mass. Nuclear membrane still intact. Note the hyaline area round the chromosomes imbedded in the nucleolar substance. Fig. 7. Late prophase. The nuclear membrane broken at the polar regions and also at the equatorial regions. Fig. 8. Metaphase. Note the cytoplasmic accumulation outside the poles of the spindle. Fig. 9. Early anaphase. The nucleolar substance divided into two plates. Fig. 10. Polar view of metaphase. 24 Chromosomes seen inside the nucleolar mass, each with a thin hyaline portion round it (from microtome section). Fig. 11. Early anaphase. Two rows of chromosomes within a common matrix of nucleolar substance. Fig. 12. Late anaphase. Fig. 13. Later stage with vacuole developed inside the spindle. Fig. 14. Telophase. Fig. 15. Daughter nuclei organised. Figs. 14 and 15, $\times 780$; the rest, $\times 1150$.

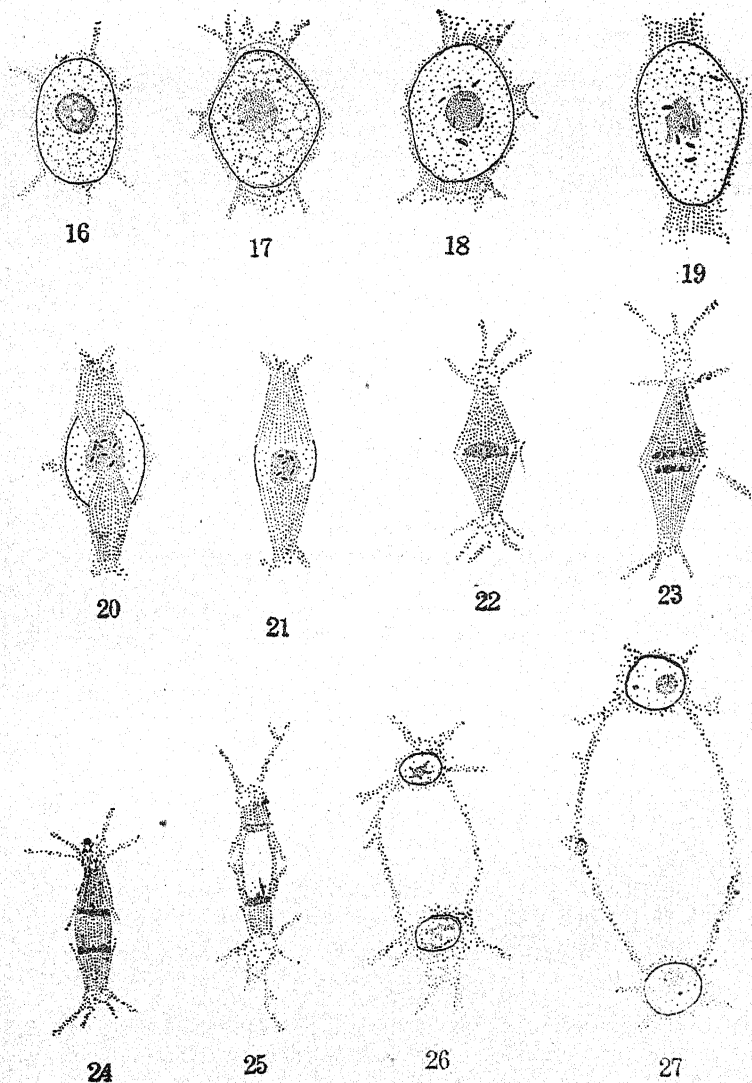
the spindle (Text-fig. 9). The hyaline space which was seen round each chromosome during late prophase and metaphase (Text-figs. 5-8, 10) is not seen during anaphase (Text-figs. 9, 11). The chromosomes at the same time appear thicker than during the previous stages (Text-fig. 11). The disappearance of the hyaline space round each chromosome and the sudden increase in the thickness of the chromosomes suggest that the hyaline area very probably represents some portion of the chromosomes which is not chromatic during prophase and metaphase, but becomes chromatic at the beginning of anaphase and takes up the stain with the result that the chromosome appears thicker and without any hyaline space round it. The two daughter plates then move still further towards the poles; and at late anaphase the plates still remain flat but the whole plate becomes darker and the distinction between the darkly staining chromosomes and the grey nucleolar substance is soon lost (Text-figs. 12, 13).

Telophase.—At the beginning of telophase the daughter plates become compressed laterally and appear somewhat twisted (Text-fig. 14). The nucleolus appears later (Text-fig. 15), but its exact origin could not be determined.

Spirogyra sp.

The resting nucleus has a prominent nucleolus and a faintly staining reticulum (Text-fig. 16). A number of vacuoles of varying sizes is seen inside the nucleolus.

Prophase.—At the beginning of prophase, the nucleus enlarges and darkly stained granules become prominent at the corners of the meshes of the reticulum (Text-fig. 17). At a later stage, six short thread-like structures are seen in the outer-nucleus (Text-fig. 18). At this stage the nucleolus is still quite intact. At a still later stage, its outline becomes slightly broken up and the vacuoles are no longer seen inside it. The nucleolus then disintegrates into a mass of granular substance. The thread-like structures become condensed into short thick darkly stained chromosomes. These chromosomes are seen crowded round the nucleolar substance and later on to



Text-figs. 16-27. *Spirogyra* sp.—Fig. 16. Resting nucleus. Note the vacuoles inside the nucleolus. Fig. 17. Early prophase. Chromatic granules seen in the outer nucleus. Polar caps formed in the cytoplasm at the poles of the nucleus. Fig. 18. Six chromosomes fully organised in the outer nucleus and gathered round the nucleolus which is still intact. Spindle fibres developed in the cytoplasm at the poles. Fig. 19. Chromosomes entering the disintegrated nucleolar substance. Fig. 20. Late prophase with all the chromosomes imbedded in the nucleolar substance and the spindle extended upto the nucleolar mass; nuclear membrane broken through at the poles, but still intact at the sides. Note the hyaline portions round the chromosomes in Figs. 20-22. Fig. 21. Later stage showing the disappearance of the nuclear membrane. Fig. 22. Metaphase. Fig. 23. Early anaphase. Figs. 24 and 25. Later stages of anaphase. Fig. 26. Telophase. Fig. 27. Daughter nuclei organised. Note the extra body beside the nucleolus in the daughter nuclei. All figs., $\times 1150$.

enter it (Text-fig. 19). At the next stage the six chromosomes are seen completely imbedded inside the nucleolar substance. Each of the chromosomes inside the granular nucleolar substance is surrounded by a narrow hyaline area as in the previous species (Text-fig. 20).

During all these stages there takes place an accumulation of cytoplasm at the poles of the nucleus constituting the polar caps. These are thinner than in *S. columbiana* (Text-fig. 17). Later, striations are developed in this cytoplasm of the polar caps and these extend into the nuclear cavity (Text-fig. 20). The nuclear membrane first becomes ruptured in the polar region as in the previous species (Text-fig. 21). The spindle is thus seen to be extranuclear and cytoplasmic in origin.

Metaphase.—The nucleolar mass becomes spread out laterally with the imbedded chromosomes arranged more or less in a plate (Text-fig. 22). The chromosomes still show the narrow hyaline areas around them. The nuclear membrane is not present at this stage.

The spindle is broadest at the equator and narrowest at the poles and, at each end of the spindle is seen a mass of diffuse cytoplasm which is faintly granular.

Anaphase.—The chromosomes and the nucleolar mass divide into two plate-like masses each with one set of daughter chromosomes inside (Text-fig. 23). These two masses move gradually towards the poles. The chromosomes at this stage do not show the hyaline areas around them, but appear thicker. A little later the chromosomes become indistinguishable from the surrounding nuclear substance which becomes more deeply stained (Text-figs. 24, 25). Vacuoles are developed inside the spindle. These vacuoles enlarge and gradually unite with one another and finally form a single large vacuole inside. The vacuole increases in size and, as a result, the spindle becomes more and more distended and barrel-shaped.

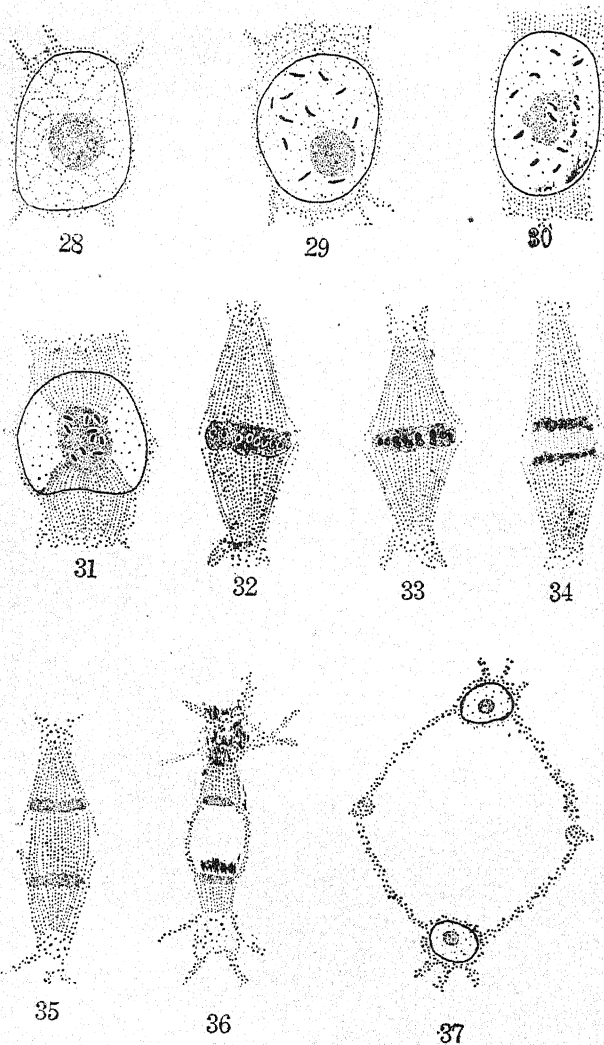
Telophase.—The flat plates of the anaphase get gradually organised into the daughter nuclei each with a nucleolus (Text-fig. 26).

In the young daughter nuclei was sometimes seen a small dark body besides the nucleolus, somewhat resembling the "*Nebenkorper*" described by Czurda (1922) in *Spirogyra setiformis*. This was not seen in any other stages.

Spirogyra Fuellibornei SCHMIDLE

The resting nucleus.—The resting nucleus has a large nucleolus with one or more vacuoles inside (Text-fig. 28). The stages of the nuclear division are very similar to those of *S. columbiana* described previously.

Prophase.—The nucleus enlarges a little at the beginning of prophase. During early prophase twelve slender slightly elongated thread-like chromosomes could be seen distributed in the outer nucleus



Text-figs. 28-37. *Spirogyra Fuellibornei* Schmidle.—Fig. 28. Resting nucleus with a large nucleolus having vacuoles inside. Fig. 29. Twelve long chromosomes seen in the outer nucleus. Nucleolus quite intact. Fig. 30. Chromosomes condensed; nucleolus beginning to break up. Fig. 31. Late prophase. All the chromosomes imbedded in the nucleolar mass. Note the hyaline region in Figs. 31 and 32. Fig. 32. Metaphase. Fig. 33. Early anaphase. Chromosomes just dividing. Figs. 34-36. Later stages of anaphase. Fig. 37. Daughter nuclei organised. Fig. 37, $\times 780$; the rest, $\times 1150$.

(Text-fig. 29). The chromosomes of this species are longer than those of the two previous species. At the stage when the chromosomes become organised, the nucleolus is quite intact and shows a regular

outline. At a later stage, the chromosomes crowd round the nucleolus, which by this time loses its crisp outline. The nucleolus then disintegrates into a mass of granular substance. The chromosomes then enter inside the nucleolar substance. In Text-fig. 30, which represents this stage, two of the chromosomes have already entered inside the nucleolar substance. Finally all the chromosomes are found imbedded in the nucleolar substance, and each of the chromosomes has a hyaline area round it (Text-fig. 31).

The spindle is formed in the same manner as in the two previous species, and starts in the cytoplasm of the polar caps and extends into the nucleus. The polar caps are much thinner in this species (Text-fig. 29) than in the previous species.

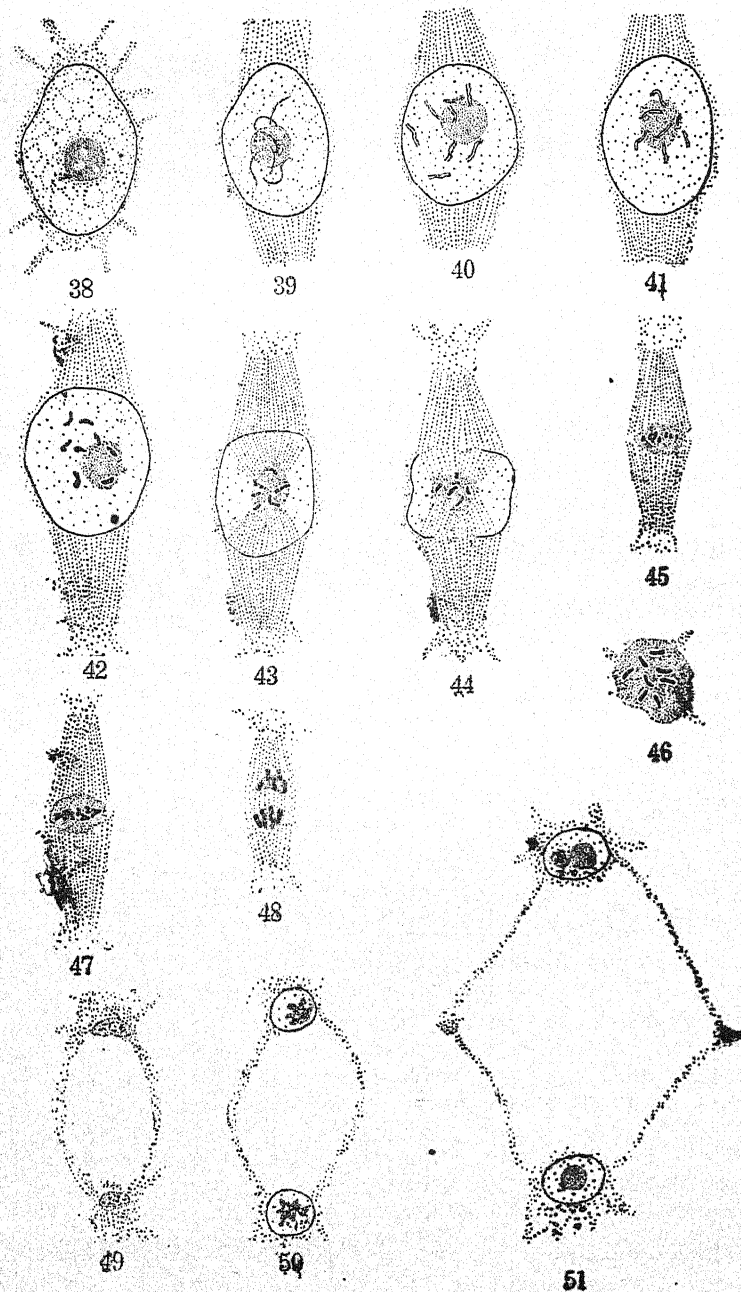
Metaphase.—The nucleolar substance with the chromosomes imbedded in it forms the equatorial plate (Text-fig. 32). Each chromosome still shows the hyaline area around it.

As in the previous forms, here also the cytoplasmic masses are seen outside the polar region of the spindle.

Anaphase.—The beginning of the anaphase is indicated by the division of the chromosomes (Text-fig. 33). The hyaline area round the chromosomes disappears and the chromosomes appear thicker. The nucleolar plate and the chromosomes inside it divide and the daughter plates move polewards. As in the other forms the daughter plates in late anaphase appear as a single mass, the distinction between the chromosomes and the nucleolar substance being lost (Text-figs. 35, 36).

Telophase.—The daughter nuclei are formed in the same way as in *S. columbiana*. A second body besides the nucleolus which was occasionally seen in *S. sp.* was not observed in this species at any time.

The material of this species was stained in Feulgen's stain also to find out how far the nucleolus could be considered as chromatic in nature. The following observations were made with material stained in Feulgen's stain. The resting nucleus remains unstained. But the slightly enlarged nucleus (just before prophase) shows a number of stained granules in the outer nucleus, while the nucleolus remains unstained. During late prophase, the chromosomes are clearly stained in the reticulum area (outer nucleus), while the nucleolus which is quite intact, remains unstained. This clearly proves that the chromatic material is found in the reticulum area and not in the nucleolus. During metaphase the chromosomes are stained red, while the nucleolar matter is not stained at all. During anaphase, when the chromosomes and the nucleolar substance become indistinguishable, the whole plate takes on a light red stain. Thus throughout nuclear division, the chromosomes show a positive reaction to Feulgen's stain, while the nucleolus shows a negative reaction. This chemical evidence clearly shows that the chromosomes are not derived from the nucleolus. These observations fully agree with those of Geitler (1935 *a*, p. 13; 1935 *b*, p. 274) and of Suematsu (1936, p. 38).



Text-figs. 38-51. *Spirogyra paraguayensis* Borge.—Fig. 38. Resting nucleus: vacuoles seen inside the nucleolus. Fig. 39. Early prophase showing long thread-like structures in the outer nucleus. Figs. 40 and 41. The thread-like structures

condensed into long chromosomes. Note the longitudinally split appearance of the chromosomes; the nucleolus still intact. Fig. 42. Mid-prophase; the chromosomes further condensed and with the split appearance lost; nucleolus breaking up and some chromosomes seen entering it. Eight chromosomes are seen. Figs. 43 and 44. Late prophase. Chromosomes already entered into the nucleolar substance; spindle extended upto the nucleolar mass. Fig. 45. Metaphase. Fig. 46. Polar view of metaphase showing eight chromosomes. Fig. 47. Early anaphase showing the chromosomes dividing. Note the hyaline area round the chromosomes imbedded in nucleolar substance in Figs. 42-46. Fig. 48. Mid-anaphase, note the thickened chromosomes and the absence of the hyaline area round them. Fig. 49. Early telophase. Fig. 50. Late telophase showing the stellate mass inside the daughter nuclei. Fig. 51. Daughter nuclei organised. All figs., $\times 1050$.

Spirogyra paraguayensis BORGE

The resting nucleus.—The resting nucleus as in the other species contains a large nucleolus. The nucleolus often contains one or more vacuoles inside it. The outer nucleus appears quite clear (Text-fig. 38).

Prophase.—At the approach of prophase, cytoplasm accumulates at the polar regions of the nucleus. In the outer nucleus there appear long slender thread-like structures, which are much longer than those met with at this stage in the three previous species (Text-fig. 39). These thread-like chromosomes become more definite at a later stage, when they take up stain more deeply. The chromosomes in this species are larger than those of the previous ones and show more details in their structure. They appear to be split longitudinally (Text-figs. 40, 41). The nucleolus is homogeneous at this stage, but still shows its original regular outline. The long chromosomes later on contract a little and approach the nucleolus and are later on seen very close to it. The nucleolus then begins to lose its sharp outline and shape, and becomes disintegrated into a mass of granular substance. Some of the chromosomes could be seen at this stage having one of their ends in intimate contact with the nucleolus appearing as though entering into it (Text-fig. 42; Pl. I, Fig. 15). Eight chromosomes could be counted at this stage. Finally in late prophase all the chromosomes are found completely imbedded inside the nucleolar substance (Text-figs. 43, 44). In this alga also a hyaline area is seen round each chromosome.

The spindle is formed in the same way as in the other species, and starts in the cytoplasm of the polar caps and extends into the nuclear cavity (Text-figs. 43, 44).

Metaphase.—In metaphase the granular nucleolar mass becomes flattened and the chromosomes are arranged in it in a plate (Text-fig. 45). As in the other forms the spindle is broadest at the equator and narrowest at the poles, beyond which there is an accumulation of cytoplasm. In a polar view of the metaphase plate obtained from a transverse section of the filament, eight chromosomes were counted (Text-fig. 46; Pl. II, Fig. 9). This number agrees with that obtained in late prophase stages. The hyaline area is still clearly seen round each chromosome.

Anaphase.—In anaphase the chromosomes divide first (Text-fig. 47). The hyaline areas round the chromosomes are no longer seen and the chromosomes appear thicker than during metaphase. Unlike the

condition seen in the other forms during anaphase, the chromosomes remain distinct from the stained nucleolar substance (Text-fig. 48). The spindle between the daughter plates as in the previous species becomes hollow and bulges out laterally.

Telophase.—During early telophase small dark bodies, short and rod-shaped or slightly longer, could be seen in the dark plates at the poles (Text-fig. 49). At this stage the nuclear membrane of the daughter nuclei are not yet organised and the nucleolar substance is still persisting. At a later stage the nuclear membrane is formed and inside this can be found a stellate darkly staining mass as in the other species (Text-fig. 50). Finally the two daughter nuclei are organised and a nucleolus could be seen in each (Text-fig. 51).

DISCUSSION

Four species were examined in the present investigation, *Spirogyra columbiana*, *Spirogyra* sp., *S. Fuellibornei* and *S. paraguayensis*. In two of the species, viz., *S. Fuellibornei* and *S. paraguayensis*, the outer nucleus remained unstained in iron-haematoxylin while in the remaining two species, viz., *S. columbiana* and *S. sp.*, outer nucleus took up a light stain, and a definite reticulum with somewhat darkly staining granules at the corners of the meshes could be seen very clearly. Geitler investigated three species, *S. crassa*, *S. sp.* and *S. setiformis* in 1930 and one more species, *S. X*, in 1935. He used paracarmine as stain and mounted the material in Venetian turpentine. He found that in *S. setiformis* and *S. X*, the outer nucleus did not take up the stain, while in the remaining two species, viz., *S. crassa* and *S. sp.*, a number of darkly staining granules was found in the outer nucleus. These dark bodies were considered by him as chromocentres. It is not clear why in some of the species the outer nucleus remains unstained. Geitler (1930, p. 96) suggests that in these species the chromatin material in the outer nucleus is very probably masked by some substance and so remains unstained.

During early prophase, in all the four species examined by the author, the chromosomes become organised in the outer nucleus, while the nucleolus is quite intact and retains its sharp outline. The chromosomes are at first scattered in the outer nucleus, but at a slightly later stage are found gathered round-about the nucleolus, which is still quite intact (Text-figs. 3, 18, 29, 40). But very soon after this, the outline of the nucleolus begins to break up and the body of the nucleolus shows signs of disintegration. A little later, the nucleolus completely loses its sharp outline and its contents become more or less a mass of granular substance. The chromosomes, which had gathered round the nucleolus previously, are then seen entering into the granular nucleolar material. Finally all the chromosomes are seen completely imbedded inside the granular nucleolar mass (Text-fig. 7, 20, 31, 43). From the above it may be seen that a definite reticulum is present in the outer nucleus as in the higher plants, and that from this reticulum the chromosomes are gradually organised. That the chromosomes are formed from the outer nucleus and not from the nucleolus can be

clearly seen from the fact that the chromosomes are completely organised in the outer nucleus while the nucleolus is still quite intact and long before it begins to break up.

These observations of the author fully agree with those of Geitler (1930, 1935 *a*) made on the four species of *Spirogyra* investigated by him. He found that the chromosomes are organised in the outer nucleus while the nucleolus is still quite intact. The nucleolus then breaks down into a granular mass and the chromosomes enter into the nucleolar substance and are finally imbedded inside the granular nucleolar mass. He comes to the conclusion that the chromosomes take their rise from the outer nucleus and not from the nucleolus. He states that if one should miss the stage where the chromosomes are formed in the outer nucleus while the nucleolus is still intact and should see all the other stages, one could easily get the wrong impression that the chromosomes are formed first inside the nucleolus and then migrate outwards into the outer nucleus.

Stolley (1930, p. 929) stated that a mere morphological study of the nucleus was not enough and that a study of its chemical nature was necessary to decide the question of the origin of the chromosomes. A number of authors have studied the effect of Feulgen reaction on the nuclei of *Spirogyra*. Petter (1933), Shinke and Shigenaga (1933) and Yamaha (1935) found that the nucleus of *Spirogyra* did not show any positive reaction to Feulgen's stain. But Geitler (1935 *a, b*) who used the Feulgen's stain for a number of species found that in almost all of them the nucleolus in the resting nucleus remained quite unstained, while some granules (chromocentres) were stained in the outer nucleus. He found that these granules in the outer nucleus later on developed into chromosomes. From this he comes to the conclusion that the nucleolus does not contain any chromatic material and that all the chromatic material is lodged in the outer nucleus (reticulum) and that the chromosomes are derived from this outer nucleus (reticulum) as in the higher plants. Yamaha and Suematsu (1938) saw in another species of *Spirogyra*, chromocentres in the outer nucleus showing a positive reaction to Feulgen's test, while the nucleolus showed a completely negative reaction. Suematsu (1936) tried the stain on some Japanese species of *Spirogyra* and found that the nucleolus showed a negative reaction while the chromatic granules and chromosomes showed a positive reaction.

In the present investigation, the author used Feulgen's stain for one species, viz., *S. Fuellibornei*. He found that in the resting nucleus both the nucleolus and the outer nucleus remained unstained. But at the beginning of prophase some granules in the outer nucleus were stained, while the nucleolus remained unstained. During the later stages of prophase, the chromosomes showed a definite positive reaction to the stain, while the nucleolus, which was still quite intact, showed a completely negative reaction. The author's observations clearly showed that the chromosomes were derived from the reticulum and not from the nucleolus, and fully agree with those of Geitler (1930; 1935 *a, b*), Suematsu (1936) and Yamaha and Suematsu (1938).

SUMMARY

A detailed account of mitosis in four species of *Spirogyra* is given in the paper.

The resting nucleus in all the four species contains a large nucleolus which takes a deep stain. The outer nucleus (reticulum) remains unstained in *S. Fuellibornei* and *S. paraguayensis*, but a lightly staining reticulum is seen in *S. columbiana* and *S. sp.* This reticulum is in no way different from that of the higher plants.

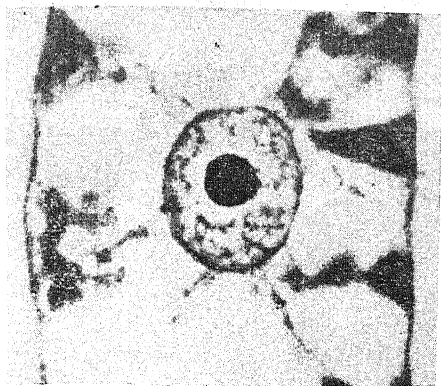
During prophase the chromosomes are formed in the outer nucleus while the nucleolus is still intact and has a sharp outline, showing clearly that they are formed from the outer nucleus (reticulum) and not from the nucleolus. About mid-prophase the nucleolus breaks down into a granular homogeneous substance, in which condition it persists throughout mitosis. During late prophase the chromosomes gradually enter the nucleolar substance and towards the end of prophase are seen completely embedded in it.

One of the four species, viz., *Spirogyra Fuellibornei*, was stained in Feulgen's stain. The reactions of the nuclear structures during division shows (1) that the nucleolus does not contain any chromatin material, and (2) that the chromosomes are derived not from the nucleolus but from the outer nucleus (reticulum). It is concluded that the nucleus of *Spirogyra* is not fundamentally different from that of the other green algæ or of the higher plants.

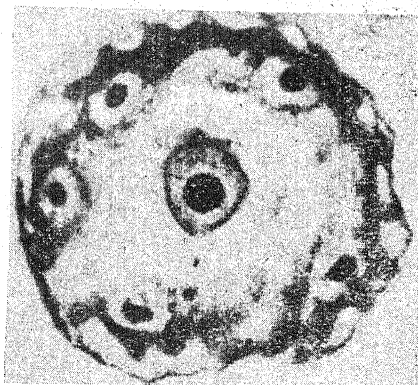
In all the four species, during late prophase, a thin hyaline area is seen round each of the chromosomes which are imbedded in the nucleolar substance. This hyaline area persists during metaphase. But during anaphase, this hyaline area is no more seen. On the other hand, the chromosomes appear definitely thicker. Whether this hyaline area represents the matrix of the chromosomes which becomes chromophilic towards the end of metaphase could not be decided with certainty.

The spindle is cytoplasmic in origin and arises in the cytoplasm of the polar caps and extends later into the nuclear cavity.

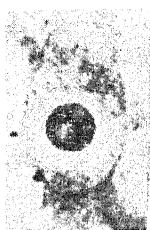
The author wishes to express his great indebtedness to Prof. M. O. P. Iyengar, M.A., Ph.D. (Lond.), F.L.S., for his constant guidance and help during the course of this investigation. His thanks are also due to the authorities of the University of Madras for the award of a research scholarship during the tenure of which the present investigation was carried out.



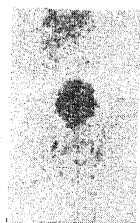
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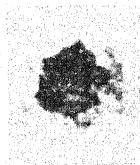
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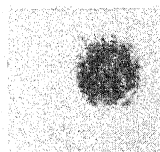
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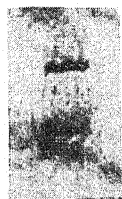
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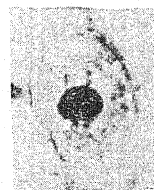
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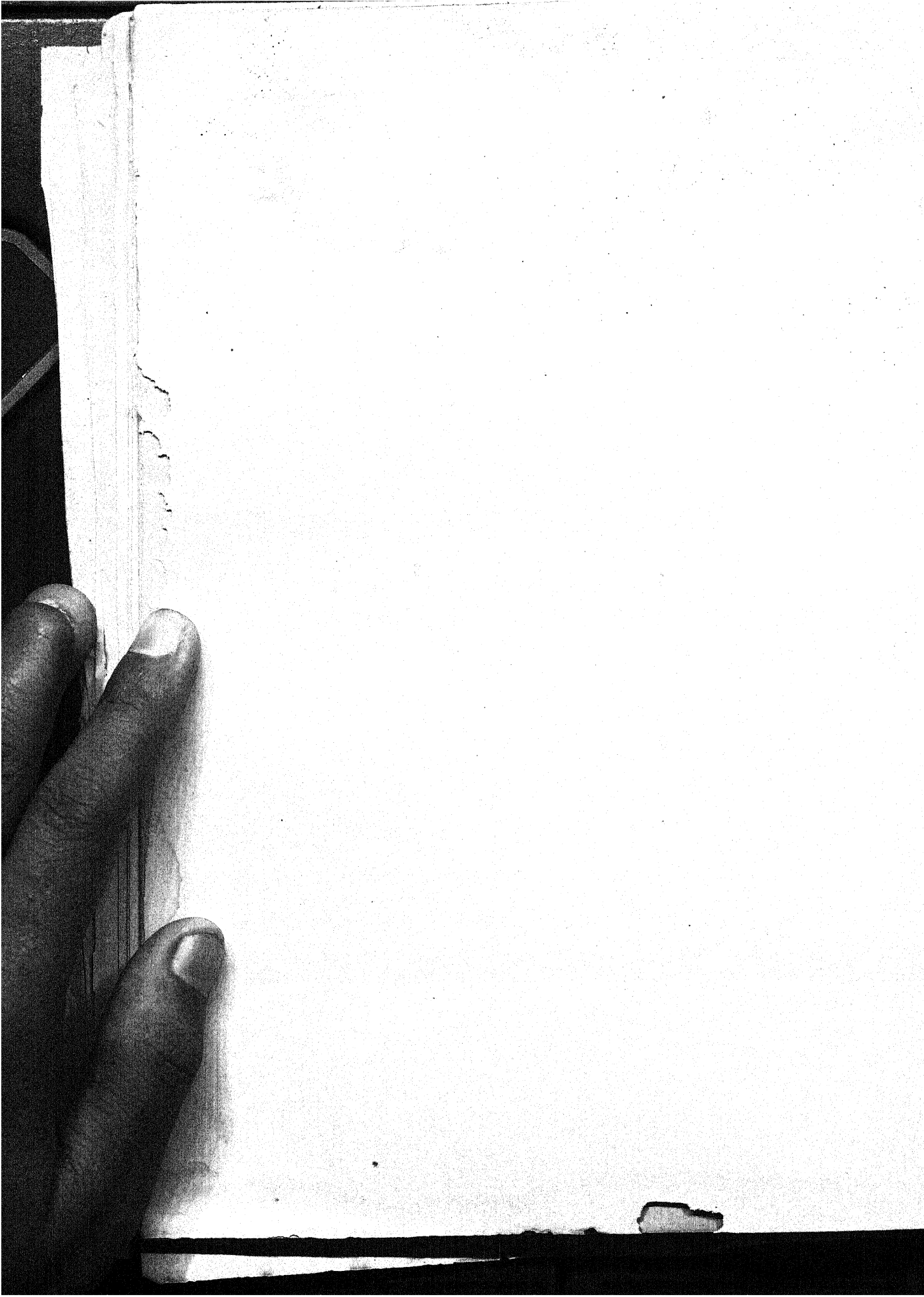


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EXPLANATION OF THE PLATE

Figs. 1, 2, 8, 9, 11-13, 15 and 16 are from microtome sections, while the rest are from whole mount preparations.

- Fig. 1. *Spirogyra columbiana*, longitudinal section of filament showing a resting nucleus with a well-defined reticulum.
- Fig. 2. *Spirogyra columbiana*, transverse section of filament showing the nucleus and the cytoplasmic strands.
- Fig. 3. *Spirogyra columbiana*, resting nucleus; note the vacuoles inside the nucleolus.
- Fig. 4. *Spirogyra columbiana*, chromosomes arising in the outer nucleus.
- Fig. 5. *Spirogyra columbiana*, late prophase showing chromosomes imbedded in the nucleolar substance; note the hyaline portion round each chromosome.
- Fig. 6. *Spirogyra columbiana*, metaphase.
- Fig. 7. *Spirogyra columbiana*, early anaphase.
- Fig. 8. *Spirogyra columbiana*, polar view of metaphase.
- Fig. 9. *Spirogyra paraguayensis*, polar view of metaphase.
- Fig. 10. *Spirogyra columbiana*, late anaphase.
- Fig. 11. *Spirogyra paraguayensis*, chromosomes organized in the outer nucleus while the nucleolus is still intact.
- Fig. 12. *Spirogyra paraguayensis*, chromosomes organized in the outer nucleus while the nucleolus is still intact.
- Fig. 13. *Spirogyra paraguayensis*, early prophase showing long thread-like chromosomes in the outer nucleus.
- Fig. 14. *Spirogyra columbiana*, telophase with the stellate dark mass in each daughter-nucleus.
- Fig. 15. *Spirogyra paraguayensis*, nucleolus breaking up and the chromosomes entering the nucleolar substance.
- Fig. 16. *Spirogyra paraguayensis*, chromosomes organized in the outer nucleus while the nucleolus is still intact.
- Fig. 8 $\times 1,200$; Fig. $\times 1,040$; the rest $\times 900$.